The Chinchilla Microdialysis Model for the Study of Antibiotic Distribution to Middle Ear Fluid

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ABSTRACT

In cases of slow or limited penetration of an antibiotic to the site of infection such as in acute otitis media (the middle ear), plasma levels of the agent may not reflect the concentrations that are relevant in determining clinical outcome. There is a need for a model that allows prediction of the time-course of unbound, pharmacologically active drug levels in middle ear fluid (MEF). This article introduces microdialysis as a sampling tool to measure unbound antibiotic concentrations in the MEF of the chinchilla, and briefly summarizes the results of studies of MEF penetration of a cephalosporin, a macrolide, and a ketolide antibiotic using this technique. The general concurrence of preliminary results of the chinchilla studies with clinical findings suggests that the chinchilla microdialysis model may be useful in predicting efficacy in patients.

KEYWORDS: microdialysis, antibiotic, acute otitis media, middle ear fluid

INTRODUCTION

Acute otitis media (AOM) is an inflammatory process of the middle ear caused by bacterial infection, typically following a viral infection of the upper respiratory tract. It is one of the most frequent infectious diseases in children and accounts for a large number of pediatric office visits.[1-3] AOM is caused by blockage of the eustachian tube, a channel lined with mucus membranes between the middle ear and the nasopharynx. When this tube becomes swollen during a cold, allergy, or upper respiratory infection, any bacteria that are present in the middle ear will lead to accumulation of fluid in the middle ear. The buildup of purulent fluid (pus) causes pressure, pain, and redness of the eardrum. Untreated AOM may cause the eardrum to rupture, causing ear drainage, or turn into serous otitis media (SOM), where pus and mucus remain in the middle ear for weeks or months and lead to serious complications.

There are many antibacterial agents used for treating AOM in infants and children. Beta-lactam antibiotics and macrolides are 2 of the main classes of antibiotics that are used in the treatment of AOM.[1,2,4-6] However, their efficacy is being compromised by the development of resistant strains of the infecting organism. An additional reason for the limited success of antibiotic therapy is that current treatment is mostly empirical. The choice of an antibiotic should be based on the likely causative microorganism and the drug's pharmacokinetics, pharmacodynamics, and pharmaceutical properties.[1,4] Thus, a drug with broad antibacterial spectrum and high potency against resistant strains is desirable. An ideal therapeutic agent should be a drug that exhibits potent antibacterial activity, is formulated in liquid form with acceptable taste (or can be given with food), and can be dosed once or twice a day.

To better predict the efficacy of antibiotic therapy in treating pediatric patients with AOM, Craig and Andes[7] developed a model that considered the percentage of time that the serum concentration of the agent remained above the minimum inhibitory concentration (MIC) during each dosing interval, and the peak antibiotic concentration in the middle ear fluid (MEF)/MIC ratio as 2 predictors. They found that when serum antibiotic concentrations exceeded the MIC for 40% to 50% of the dosing interval, an average cure rate of 80% to 85% was observed. When the antibiotic concentration was above MIC for 60% to 70% of the dosing interval, a bacteriologic cure rate of close to 100% was seen. In addition, if the peak concentration in MEF/MIC ratio was between 3.2 and 6.3, an 80% to 85% bacterial efficacy was seen. When this ratio exceeded 10, 100% bacterial eradication was observed.[7]

This model, however, does not consider that plasma levels of the antibiotic may not reflect the concentrations at the site

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of the infection—the MEF. Thus, where there is a slow equilibrium achieved between MEF and plasma, predictors of cure based on plasma concentrations as a surrogate for MEF levels, may be subject to error. This is particularly true if the MEF levels decline more slowly than those in the plasma, and/or there is a slower accumulation of antibiotic levels in the MEF than in plasma during continued dosing following initiation of therapy. Clearly, there is a need for a model that allows prediction of the time-course of MEF antibiotic levels based on knowledge of those in the plasma. It would be especially helpful if the unbound, pharmacologically active levels of the antibiotic could also be predicted from this model. Such a model may be derived from continuous measurements of plasma and MEF levels of antibiotic in an animal model that reflects what occurs in a pediatric patient. This article describes the application of microdialysis as a sampling tool to characterize unbound antibiotic concentrations in the MEF of the chinchilla and provides findings obtained in studies of MEF distribution kinetics for a beta-lactam, a macrolide, and a ketolide antibiotic using this methodology.

MICRODIALYSIS SAMPLING OF CHINCHILLA MIDDLE EAR FLUID

The concentration of antibiotics in plasma can easily be determined by traditional pharmacokinetic sampling techniques. However, since the amount of MEF produced in humans is small and variable, it is difficult to use conventional discrete sampling techniques to obtain a full PK/PD profile of antibiotics in the middle ear. Moreover, since only free (unbound) drug is effective against bacteria, the need for determining free antibiotic concentration in MEF adds more difficulties to the measurement. In vivo microdialysis can be used to solve these problems.

Microdialysis

Microdialysis involves the insertion of a miniature probe into a selected fluid or tissue in vivo.[8] The microdialysis probe is composed of a short length of semipermeable hollow fiber dialysis membrane connected to an inlet and outlet tubing. A perfusion solution (perfusate) is pumped slowly through the probe and collected for analysis. The perfusate, which is isotonically matched to the surrounding medium to minimize osmotic pressure differences, often contains a calibrator that diffuses from the solution into the tissue or fluid being sampled; the loss of this "retrodialysis" calibrator is used to estimate the recovery of the analyte (eg, antibiotic) from the sampled fluid (eg, MEF) by dialysis. The perfusion flow rate used in microdialysis generally ranges from 0.1 to 5.0 μL/min. Exchange of substances occurs across the dialysis membrane according to the concentra-

tion difference. The membrane is permeable to small molecules but not to macromolecules such as proteins. Thus, microdialysis measures "unbound" drug levels, providing an index of pharmacologically active concentrations. The dialysate, which is the solution exiting the probe, can be collected for analysis. Microdialysis probes can be designed in a variety of shapes and sizes, to be suitable for sampling several tissue spaces and fluids.

The basic setup of a microdialysis system and how it may be interfaced with a chinchilla has been described previously.[9,10] The system consists of a microdialysis probe, an animal, a perfusion pump, inlet and outlet tubing, and a (refrigerated) microfraction collector. For online microdialysis, the fraction collector is omitted and the dialysate is directed to a high-performance liquid chromatography (HPLC) instrument with an appropriate detector. Where more than one site (right and left middle ear spaces, for example) is being studied, a digital sequence programmer is added to allow alternate injection of the dialysates from sample loops onto an online analytical system. A turntable may be used where freely moving animals are studied, to prevent tangling and twisting of the inlet and outlet tubing by the animal. A major advantage of this technique in assessing the degree of penetration of an antibiotic into MEF is that continuous monitoring of MEF drug levels for several hours after dosing allows for characterization of the rate of equilibration and the changing ratio of MEF/plasma levels of the antibiotic.

The Chinchilla Model

The tympanic bulla (middle ear cavity) of the chinchilla is ~25 mm long in the anterior-posterior direction, 22 mm high, and 12 mm wide. It is located posterior to the brain and is easy to access from the scalp without damaging the brain. The total volume (V) of the bulla is between 1.52 and 2.09 mL,[11-13] which is large compared with other animals. The surface area (SA) of the bullae has been estimated to be 14.4 cm^2. The surface area to volume ratio of an extravascular space is important in determining drug concentration behavior in this space. The chinchilla middle ear SA/V ratio is 6.9, which is very close to that of humans.[13]

AOM in the chinchilla middle ear model closely parallels the continuum of human AOM. The inoculation of bacteria into the chinchilla middle ear causes a replication of local infection with polymorphonuclear leukocyte (PMNL) infiltration into the subepithelial space of the middle ear mucosa. Epithelial metaplasia, subepithelial edema, and MEF volume increase during the first week after inoculation, followed by gradual resolution of inflammation over the succeeding 8 to 10 weeks. In addition, a chinchilla model with middle ear pneumococcal infection can be produced by instilling a very small amount of inoculum into the

middle ear, confining the infection to that site.[13] Well-controlled conditions can reliably produce AOM in chinchillas with low interanimal variability.[13-15]

In our studies, 1- to 2-year old chinchillas, weighing 400 to 600 g, are used. During the study period, the animals are conscious and are housed in a chamber that permits free movement of the animal and access to food and water. The chamber is rotated in a direction that counters the movement of the animal, thereby preventing twisting of the microdialysis lines and the vascular catheters that are typically employed for drug dosing and blood sampling.

The experiments allow for continuous monitoring of the antibiotic concentrations in MEF at intervals of 10 to 30 minutes, depending on the chromatographic run time of the antibiotic studied and whether concentrations in one or both ears are being measured. Calculation of the unbound concentrations of the antibiotic in MEF is based on microdialysis recovery, determined using a validated retrodialysis calibrator added to the perfusate.[16]

DISTRIBUTION OF ANTIBIOTICS BETWEEN PLASMA AND MIDDLE EAR FLUID IN THE CHINCHILLA STUDIED WITH MICRODIALYSIS

In order to examine the penetration of antibiotics into MEF, studies are undertaken in chinchillas, which are surgically prepared to allow the antibiotic to be given intravenously via the femoral or jugular vein and to permit blood sampling via the femoral artery. Following bilateral surgical obstruction of the eustachian tubes, instillation of artificial MEF (AMEF), and placement of the vascular catheters, the bullae of both the right and left middle ears are implanted with microdialysis probes. Where infected animals are to be studied, 50 CFU of *Streptococcus pneumoniae* 23 F-Pelton, No. 365, are inoculated into the middle ear cavity 24-hours after instillation of the AMEF.[9,10] Although the data presented in this article were from noninfected animals, the procedures performed on infected chinchillas are summarized in Figure 1.
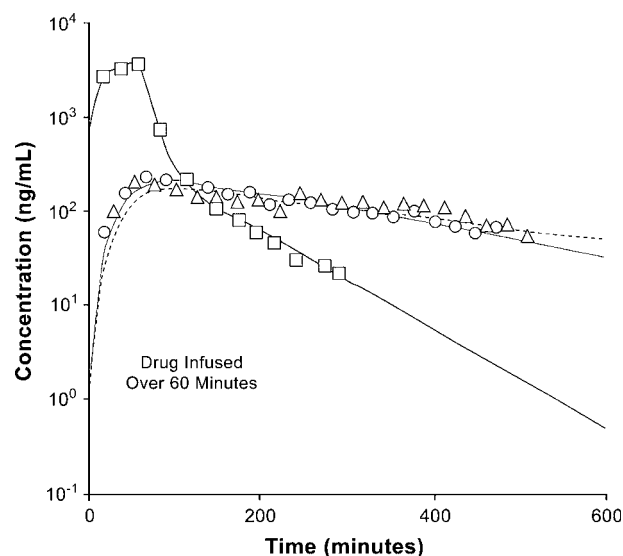
Cefdinir

The objectives of this study[17] were to quantitatively examine the distribution kinetics of cefdinir into MEF using the freely moving chinchilla microdialysis model, and to provide a basis for dosing guidelines for cefdinir in pediatric patients with AOM caused by penicillin-resistant *S pneumoniae*. Single intravenous infusions, as well as multiple intravenous infusions every 4 hours, were employed. Animal preparation and microdialysis have been described elsewhere.[9,10] An online microdialysis-HPLC-UV system was established for the analysis of cefdinir and cefixime (the retrodialysis calibrator) in dialysates. Plasma samples collected from the
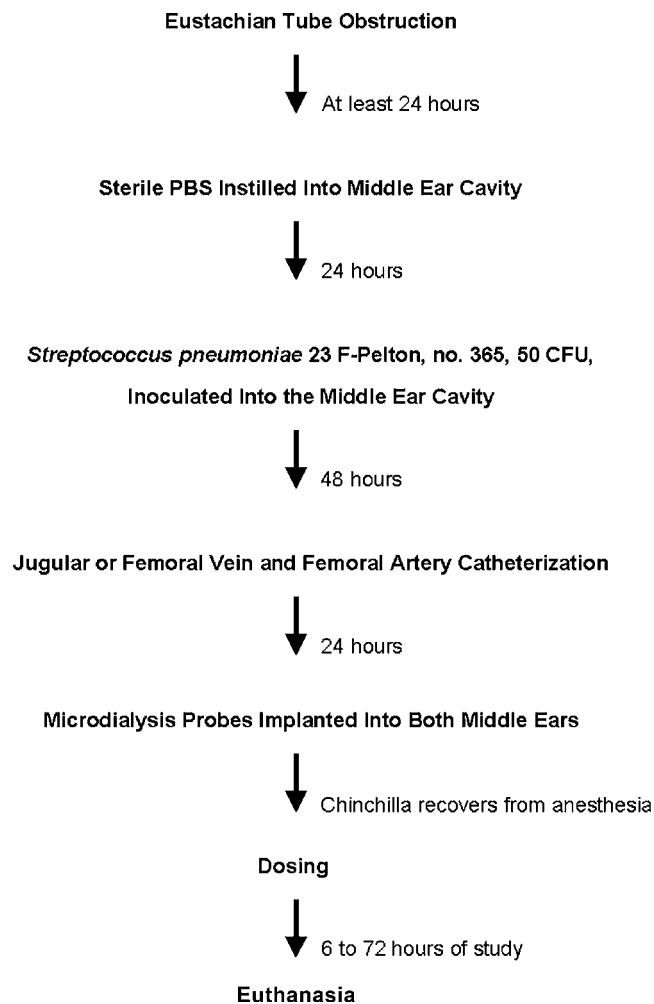
Figure 1. Surgical procedures performed on infected chinchillas prior to and during the microdialysis study period.[10]

dosing studies were analyzed for cefdinir using an offline liquid chromatography (HPLC)–mass spectrometry (MS)–MS method.

Twenty-one noninfected chinchillas were given single doses of cefdinir in doses of 1.5, 5, or 7 mg/kg, administered intravenously at a constant rate over a 60-minute period. Plasma was assayed for drug using HPLC–MS–MS. Dialysates from probes in both ears were alternately collected and injected onto the HPLC-UV system for analysis of unbound concentrations of cefdinir. Figure 2 shows plots of the observed total plasma concentrations and MEF levels of cefdinir in one chinchilla receiving a single dose of the drug as a 60-minute infusion of 5 mg/kg, and a simulation of the plasma concentration-time profile using fitted parameters for this animal. Cefdinir exhibited a longer mean half-life in MEF (134 to 207 minutes) than that in plasma (28 to 58 minutes) in the 3 dose groups. No significant difference was found in the systemic (plasma) clearance across the 3 doses. The ratio of the area under the unbound concentration curve in MEF (AUCmef) over the area under the unbound concentration curve in plasma (AUCplasma,u) was (mean ± SD)
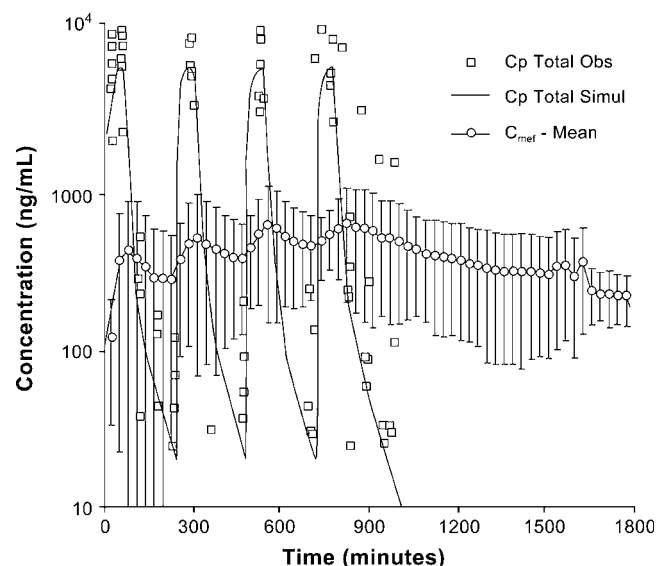
Figure 2. Observed total plasma concentrations (open squares) of cefdinir in a chinchilla receiving a single dose of the drug as a 60-minute infusion of 5 mg/kg.[17] A simulation of the plasma concentration-time profile in this animal is shown as the solid line through the open squares. The C_{mef} data for right and left ears are also plotted (open circles and triangles, respectively).

Figure 3. Observed total plasma concentrations (Cp total obs) of cefdinir in 7 chinchillas receiving the drug as 60-minute infusions of 7 mg/kg every 4 hours for 4 doses.[17] A simulation of the plasma concentration-time profile generated using the means of the 4 parameters (volume and rate constants) derived from the 2-compartment model is also shown (Cp total simul). The means and SD of the unbound C_{mef} data, grouped in 30-minute intervals, are also plotted (C_{mef}).

0.66 ± 0.66, 0.49 ± 0.31, and 0.69 ± 0.39 in the 1.5-, 5-, and 7-mg/kg dose groups, respectively.[17]

Fourteen noninfected chinchillas were studied using 2 multiple dosing regimens. The animals received intravenous cefdinir (2 or 7 mg/kg) every 4 hours for 4 doses. Each dose of cefdinir was infused intravenously at a constant rate over 1 hour. Dialysates from the microdialysis probes in both ears were alternately collected and injected into HPLC-UV system for analysis of unbound concentrations of cefdinir. Blood samples were collected at fixed times after the initiation of the first dose via the femoral artery catheter. Plasma was analyzed for drug using HPLC–MS–MS. Figure 3 shows plots of the observed total plasma concentrations in chinchillas receiving 7 mg/kg cefdinir every 4 hours for 4 doses. The means and SD of the Cmef data are also plotted.

Although plasma levels of cefdinir reached a steady-state by the second dose, MEF concentrations of this cephalosporin showed an increasing trend during continued dosing. The differences between first and fourth doses were significant in both regimens for both left and right ears. This accumulation of cefdinir in MEF, expressed as the ratio of the Cmax 4th (maximum concentration during the fourth dose) to Cmax 1st (maximum concentration during the first dose), averaged (right and left ears combined) 2.3 and 2.6 for the 2 mg/kg and 7 mg/kg regimens, respectively (significantly different from unity, $P < .05$). This slow approach to steady-state suggests that the use of a loading dose of 2- to 3-fold higher than the maintenance dose would hasten the approach to steady-state MEF levels in this model. No significant

difference was found in the dose-normalized AUC in plasma or in MEF between the 2 dose groups, suggesting linear pharmacokinetics in this dose range.[17]
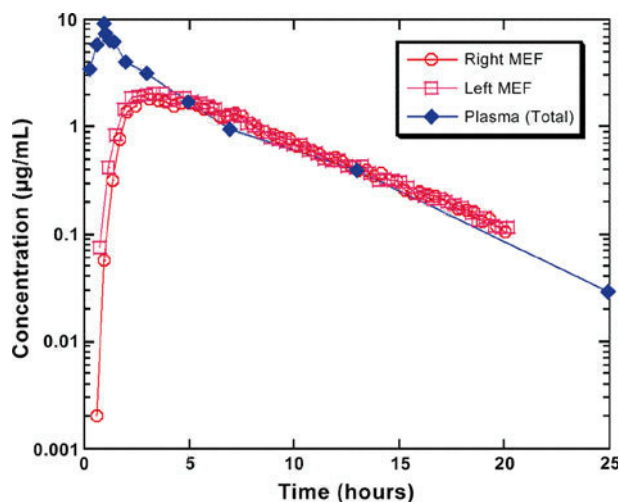
Clarithromycin

It is believed that successful antibiotic therapy of patients with otitis media requires middle ear penetration of the antibiotic, resulting in sufficient unbound levels in MEF to kill bacterial pathogens. However, some antibiotics, such as azithromycin and clarithromycin appear to concentrate intracellularly, rather than in middle ear fluid. Thus the effective concentrations that are achieved during therapy using such agents have been questioned. We performed a pilot study in the chinchilla to estimate the unbound concentration time profile of clarithromycin following intravenous doses of 30 and 50 mg/kg. The animals were noninfected controls, prepared surgically as described above and fitted with microdialysis probes in both bullae. Clarithromycin was infused intravenously at a constant rate over a period of 0.5 hour in the 30 mg/kg dosing group, and over 1 hour in the 50 mg/kg group. Plasma levels of the antibiotic were determined by traditional serial blood sampling over approximately one day. Continuous microdialysis of the MEF in both ears was performed over the same time course, with dialysates samples alternately injected onto the HPLC–MS–MS for analysis at 10-minute intervals, such that 6 dialysate samples per hour were assayed for clarithromycin.

Microdialysis recovery of the antibiotic was continuously monitored using retrodialysis of oleandomycin, which was added to the probe perfusate.

HPLC and MS instrumentation and conditions for the analysis of clarithromycin and oleandomycin in dialysates were HPLC interfaced with an API-365 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) using a turbo-ion spray source (Shimadzu, Kyoto, Japan). Mass ratios for detection were set at 748/590 amu for clarithromycin and 688/545 amu for oleandomycin. The drug of interest and its calibrator were separated using a YMC J'sphere column (Waters, Milford, MA) and a mobile phase consisting of ammonium acetate buffer, acetonitrile, and methanol at a flow rate of 0.1 mL/min. The assay of clarithromycin in plasma used 0.05 mL of matrix and was similar to that for MEF dialysates.

Calculation of the unbound clarithromycin concentrations in MEF was based on recovery. Typical results obtained are shown in Figure 4, which represents plasma and MEF data from an animal receiving an intravenous dose of 50 mg/kg of clarithromycin infused over 1 hour. Although peak unbound levels of clarithromycin in the MEF occur at ~3 hours after the infusion was stopped and are ~20% of the total plasma levels, the MEF levels in the decline phase are about twice the unbound levels in plasma (the free fraction of this drug in chinchilla plasma is ~55%). The disappearance half-life of clarithromycin from MEF appears to be controlled by its biological half-life (ie, that observed in plasma). The ratio of the AUCmef over the AUCplasma,u was (mean ± SD) 1.35 ± 0.34 and 1.41 ± 0.56 in the 30-, and 50-mg/kg dose groups, respectively.

The interpretation of measured MEF levels of an antibiotic following systemic dosing is problematic since the time course of drug in this target space reflects both penetration into the MEF and distribution back into plasma. Indeed, if the antibiotic is unstable in the MEF, this further complicates the picture. Dosing of the antibiotic directly into the middle ear cavity (bulla) of the chinchilla allows for an analysis of its elimination kinetics from this site. Characterizing the input rate when the antibiotic is given systemically in subsequent studies is then possible. Deconvolution may be used to determine the input function (rate of penetration into the MEF) if drug elimination from the MEF obeys linear kinetics. The following pilot study was performed in a chinchilla to determine the elimination characteristics of clarithromycin from MEF.

In order to evaluate the elimination kinetics of clarithromycin from the middle ear, an intrabulla dosing experiment was conducted. A 40-µg dose of clarithromycin was delivered to both middle ears of a male chinchilla in 0.8 mL of phosphate-buffered saline (pH 7.45) containing 3% bovine serum albumin (BSA). The drug/protein solution was instilled into the bulla via a 10-cm polyethylene catheter (internal diameter of 0.58 mm). Unbound middle ear clarithromycin concentrations were monitored using CMA-20 microdialysis probes (CMA Microdialysis, North Chelmsford, MA) perfused at a volumetric rate of 0.3 µL/min. Dialysate was directed to an HPLC–MS–MS for analysis over ~17 hours. Retrodialysis with a calibrator (oleandomycin) was continuously performed over the study period in both bullae to determine recovery of clarithromycin from the MEF. Analysis of both analytes was performed by HPLC–MS–MS as described above.

Figure 5 shows the drug concentration profiles in the middle ear measured by microdialysis following intrabulla dosing.
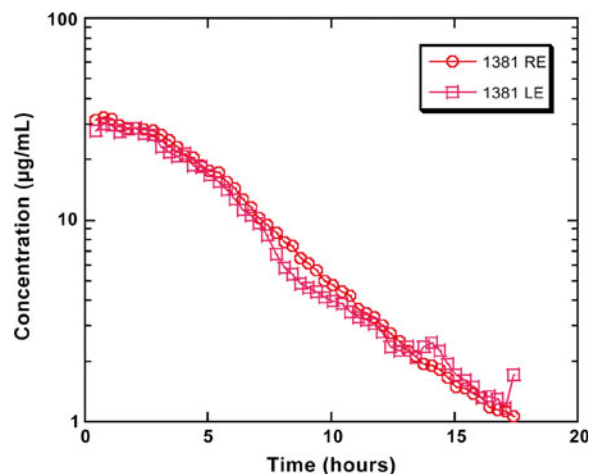
Figure 4. Observed total plasma and unbound MEF concentrations of clarithromycin in a chinchilla receiving the drug as a single constant-rate intravenous 60-minute infusion of 50 mg/kg. Plasma levels were determined by traditional serial sampling, whereas MEF levels were determined by quantitative microdialysis.

Figure 5. Unbound MEF concentrations of clarithromycin in a chinchilla receiving the drug as a single intrabulla dose of 40 µg in both middle ears. MEF levels representing a unit impulse response function were determined by quantitative microdialysis.

After ~3 hours, the clarithromycin concentration decline was approximately monoexponential, with a half-life of ~4.5 hours. The apparently longer half-life observed over the first 3 hours when the concentrations are in the range of 30 µg/mL suggests the possibility of nonlinear elimination from the MEF and would indicate that a unit impulse response function observed at doses lower than 40 µg should be evaluated if deconvolution is to be used to assess the rate and extent of entry of clarithromycin into MEF where the antibiotic is given systemically. This evaluation is currently being pursued in our laboratory.
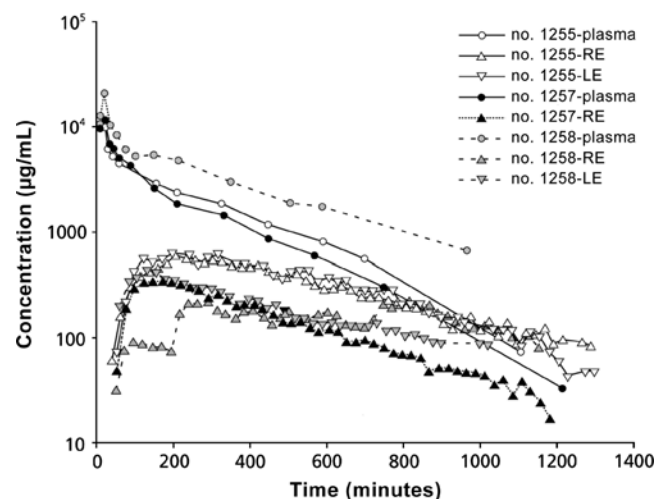
Cethromycin (ABT-773)

Recently, a novel group of 14-membered macrolides, known as the ketolides, was introduced[18-20] exhibiting increased acid stability and antibacterial potency against erythromycin-resistant bacteria.[21,22] This study[23] was undertaken to determine the extent of distribution of the ketolide antibiotic, cethromycin (ABT-773), into chinchilla MEF.

Single intravenous doses of cethromycin were given to noninfected chinchillas at 2 dose levels (25 and 50 mg/kg infused over 30 minutes) to characterize its pharmacokinetics and distribution into MEF. Blood sampling and microdialysis of MEF, using an appropriate retrodialysis calibrator, was conducted up to 24 hours postdose. The free fraction of cethromycin in plasma was determined using ultrafiltration at 37°C in freshly harvested chinchilla plasma at 1, 5, and 25 µg/mL. The free fraction was found to vary from 3.4% to 4.4%, and was independent of plasma concentration.[23]

The results demonstrated that cethromycin exhibits linear systemic pharmacokinetics in the dose range studied. Good penetration of cethromycin into the MEF was noted. Of interest, the AUC of unbound cethromycin in MEF was 3.9 ± 1.4 times the estimated unbound plasma AUC (the average unbound fraction in chinchilla plasma was found to be 4%), suggesting that cethromycin may be actively transported into MEF.[23] The possibility of ion-trapping of the protonated form of the antibiotic in MEF (which might be expected for a basic drug at a lower pH) cannot be excluded, although poststudy pH values were not measured in these MEF samples. Plasma and MEF levels of cethromycin in 3 chinchillas receiving intravenous doses of 25 mg/kg are plotted in Figure 6.

Conclusions

The results of studies of the distribution of antibiotics into chinchilla MEF performed in our laboratory show that cefdinir does not exhibit unbound levels equal to those in plasma at equilibrium. Conversely, there is evidence that macrolide and ketolide antibiotics may distribute favorably into the MEF, reaching unbound concentrations in that space

Figure 6. Observed total plasma concentrations (circles) of cethromycin (ABT-773) in 3 noninfected chinchillas (no. 1255, no. 1257, and no. 1258) receiving single intravenous doses of 25 mg/kg of cethromycin over 30 minutes. The unbound MEF levels, measured by microdialysis, are shown as upright (right ear [RE]) or inverted triangles (left ear [LE]). AUCs of unbound antibiotic in MEF were ~13% of the AUCs of total drug in plasma, but ~3.9 times the calculated AUC of unbound antibiotic in the plasma.[23]

that are higher than the corresponding plasma levels.[9,10,17,23] Whether this is a result of the existence of transporters in the middle ear epithelial mucosa is unknown.

Babl et al,[24] using an experimental model of otitis media, gave chinchillas infected with nontypeable *Hemophilus influenzae* oral doses of 30 or 120 mg/kg of azithromycin once daily for 5 days. The investigators found that the 30-mg/kg regimen produced 24-hour serum levels and AUCs similar to those seen in children receiving azithromycin in 5 to 10 mg/kg per day regimens. The ratio of total azithromycin levels (not corrected for protein binding) in MEF extracellular fluid relative to that in serum ranged from 2.3 to 4.8 just prior to dosing on days 3 and 5 in the 2 regimens. These ratios, where MEF levels of azithromycin were measured in freeze-thawed samples that included cells, were 10.6 to 18.5, consistent with high intracellular concentrations.

Because microdialysis was not employed in the studies by Babl et al,[24] it is unclear what the pharmacologically active (unbound) levels of azithromycin in MEF were, or how the time-course of these levels compared with those in serum. It would be of interest to use microdialysis to continuously monitor MEF levels of azithromycin in this model, and in the uninfected chinchilla to assess the effect of inflammation of middle ear epithelium on antibiotic penetration into the middle ear cavity.

Craig and Andes[7] reviewed the clinical literature characterizing the MEF/plasma ratios of antibiotics and have reported that, for penicillins and cephalosporins, these ratios are less

than unity in pediatric patients, whereas for clarithromycin and its active 14-hydroxylated metabolite, the ratios are greater than one. Because the timing of the samples used to determine these ratios was variable in these studies, and since total levels rather than unbound concentrations were measured, the results should be interpreted with caution. Nevertheless, there appears to be general agreement between the preliminary results of the chinchilla microdialysis studies and the clinical findings, suggesting that the chinchilla microdialysis model may be a useful predictive tool for what may be expected in pediatric patients.

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